

### **REMARKS**

In response to the election requirement, the Applicants withdraw without prejudice claims 3, 10, 19-42, 45, 46 and 48 to 51 and submit the listing of claims enclosed herewith in replacement of all prior versions. Claim 6 is rewritten to reflect correct amendments to the claim.

In this new listing of the claims:

Claims 1, 2, 6 and 7 have been amended;

Claim 8 is original;

Claim 9 has been amended;

Claim 43 has been amended;

Claim 47 is original;

Claims 53 to 57 are new and find proper support in various sections of the specification;

Claims 58 and 59 are new and correspond respectively to claims 12 and 18 presently on file; and

Claims 4, 5, 11 to 18 and 44 presently on file have been withdrawn without prejudice.

As requested by the Examiner in **paragraph 4**, a new and corrected Declaration duly signed by GIDDA SATINDER is enclosed.

In **paragraph 5**, The Examiner noted that JP 02-092220 listed in the IDS filed by the Applicants has not been provided. Accordingly, a copy of the abstract of this publication in English, and a copy of the publication in Japanese are provided herewith for the Examiner's convenience.

In **paragraph 6**, the Examiner has rejected claims 6, 14 and 44 presently on file for reading on non-elected inventions. In response to this rejection, the Examiner will note that the new set of claims has been limited to the elected invention.

In **paragraph 7**, the Examiner has objected to the specification because of the embedded hyperlinks. In response to this rejection the hyperlinks appearing on pages 17 and 18 of the specification have been deleted.

In **paragraph 8**, The Examiner has rejected claims 6 to 8 and 14 presently on file under 35 U. S. C. 112, second paragraph as being indefinite and failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. In response to this rejection, the new claims now refer to a SEQ ID from the sequence listing.

In **paragraphs 9 and 10**, the Examiner has rejected claims 1, 2, 4 to 9, 11 to 18, 43, 44 and 47 presently on file under 35 U.S.C. 112 first paragraph as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the art that the inventors, at the time the application was filed, had possession of the claimed invention, and because the specification is only enabling for a method of increasing time to flowering in *Arabidopsis* plants comprising transforming said plants with the *Arabidopsis* AtST2a genomic sequence of SEQ ID NO:1, operably linked to a promoter in antisense orientation, wherein the level of 12- or 11- hydroxyjasmonic acid are increased relative to non-transgenic plants.

In response to this rejection, the Applicants submit a new listing of the claims relating to a method of increasing time to flowering in *Arabidopsis* plants, comprising transforming said plants with the *Arabidopsis* AtST2a genomic sequence of SEQ ID NO:1, operably linked to a promoter in antisense orientation, wherein the level of 12- or 11- hydroxyjasmonic acid are increased relative to non-transgenic plants. Hence, the listing of claims is now fully supported and enabled by the description.

Moreover, the sequence described in the present application contains the motifs that are well known to be present in all soluble sulfotransferases that have been characterized so far. It is possible to find this information by doing a protein blast search at NCBI. More specifically, two domains are highly conserved.

- The first one comprises the sequence YPKSGTTW and is localized at the N-terminal of all sulfotransferases. The lysine residue of this domain has been shown to bind the sulfate donor 3'-phosphoadenosine 5'-phosphosulfate (see Marsolais, F and Varin, L. (1995) Identification of amino acid residues critical for catalysis and substrate binding in the flavonol 3-sulfotransferase. *J. Biol. Chem.*, **270**, 30458-30463.)
- The second domain, RKXXGDWKNXFT, is localized closer to the C-terminal extremity. The arginin residue of this motif is critical for the binding of the sulfate donor.
- There are also other amino acid residues that have been shown to be absolutely required for activity such as histidine 118 (numbering of the flavonol 3-sulfotransferase) which acts as base catalyst during catalysis.

It is very easy for a person skilled in the art, namely, one having expertise in protein chemistry, to find these conserved domains and to assess the sulfotransferase function to an unknown protein having these motifs.

Another reference that can be used to find the structural characteristics of sulfotransferases is: Marsolais, F. and Varin, L. (1998) Recent developments in the study of the structure-function relationship of flavonol sulfotransferases. *Chem. Biol. Interact.*, **109**, 117-122.

The Applicants also submit that knowing the conserved domains present in all soluble sulfotransferases, it is very easy to identify in databases protein sequences having sulfotransferase activity. A FASTA search in SWISSPROT using the string YPKSGTTW will retrieve a large number of sequences that have already been characterized at the biochemical level or that are predicted to encode sulfotransferases.

To search for new sulfotransferase coding sequences, a PCR approach using partially degenerate oligonucleotides targeting the two conserved domains would allow amplification of DNA fragments encompassing a large portion of sulfotransferase coding sequences. The PCR products can then be used to screen libraries to search for full-length clones. Someone skilled in the art can easily perform this molecular approach.

Finally, despite the fact that it is difficult to predict exactly the outcome of plant transformation experiments due to the random insertion of the T-DNA in transgenic plants, the characterization of a number of independent lines allow to find the one(s) expressing the transgene at an adequate level. This is true both for overexpression in sense and underexpression via antisense or RNA interference. Someone skilled in the art, would easily bypass this apparent pitfall of transgenic plant production.

In **paragraph 11**, the Examiner has rejected claims 1 and 2 presently on file under U. S. C. 102(b) as being anticipated by Krajncic et al. (1995, J. Plant Physiol. 146:754-756). In response to this rejection, claims 1 and 2 have been amended.

Hence new claims 1 and 2 are now directed to a method for modulating or inducing flowering in a plant, comprising modifying in said plant the endogenous level of at least one compound selected from the group consisting of 12-hydroxyjasmonic acid, glucoside of 12-hydroxyjasmonic acid, sulfate ester of 12-hydroxyjasmonic acid, 12-hydroxymethyljasmonic acid, glucoside of 12-hydroxymethyljasmonic acid, sulfate ester of 12-hydroxymethyljasmonic

acid, 11-hydroxyjasmonic acid, glucoside of 11-hydroxyjasmonic acid, sulfate ester of 11-hydroxyjasmonic acid, 11-hydroxymethyljasmonic acid, glucoside of 11-hydroxymethyljasmonic acid, sulfate ester of 11-hydroxymethyljasmonic acid, and mixtures thereof, wherein the endogenous level of at least one compound is modified by modulating the expression of a sulfotransferase encoded by a gene of SEQ ID NO:1.

On the other hand, the scientific article by Krajncic et al. discloses the addition of jasmonic acid solution to the nutrient solution of the experimental plants *Spirodela polyrrhiza* and does not disclose the modulation of the expression of a sulfotransferase encoded by a gene of SEQ ID NO:1.

Moreover, and as described in the application, the novelty of the present invention resides in the fact that the Applicants has demonstrated that it is not jasmonic acid which is responsible for the induction of flowering, but 12-hydroxyjasmonate. The Applicants agree that the treatment of plants with jasmonic acid can affect flowering time but submits that it will also affect other aspects of plant development. The Applicants also agree that a mutation in the jasmonate pathway will ultimately lead to the absence of 12-hydroxyjasmonate and consequently give rise to late flowering plants, but the present invention has the advantage to keep the jasmonate pathway intact. For example, a knock-out mutation of the gene encoding the enzyme Allene Oxide Synthase (AOS) results in plants which are deficient in jasmonic acid (J-H Park et al. 2002) but as stated in the research article, these plants are also defective in wound signal transduction and are more susceptible to pathogen infection.

Similar negative side effects were obtained with plants having a mutation in the genes encoding the enzyme Allene Oxide Cyclase (AOC), 12-oxo-phytodienoic acid reductase (OPR3) fatty acid desaturase (FAD3, 7 and 8) and lipoxygenase 2 (LOX2).

It has been demonstrated that jasmonic acid as well as its precursor 12-oxo-phytodienoic acid (OPDA) regulate the expression of several genes involved in the plant defense response. In addition, it has been demonstrated that the application of jasmonic acid to plants has deleterious effects on growth (such as repression of genes involved in photosynthesis, root growth inhibition and loss of chlorophyll). Similar experiments conducted with 12-hydroxyjasmonate did not give rise to similar negative side effects. This can be explained by the fact that 12-hydroxyjasmonate does not induce or repress the same genes when compared with jasmonic acid or OPDA.

To illustrate the difference between the effect mediated by jasmonic acid and 12-hydroxyjasmonate on gene expression, selected results from an mRNA profiling experiment performed with the *A. thaliana* Affymetrix DNA chips are presented in Table 1, 2 and 3. It is important to note that only selected genes are presented and that these results have not been published. The Affymetrix DNA chips comprise more than 22,000 entries and a large number of genes are clustering with the ones presented in the three Tables.

The results show clearly that jasmonic acid and 12-hydroxyjasmonate have different effects on gene expression. For example, 12-hydroxyjasmonate does not repress the expression of genes involved in photosynthesis (Table 1). Furthermore, 12-hydroxyjasmonate does not induce the expression of *THI2.1* a marker gene in the plant defense response (Table 2). A similar result with *THI 2.1* was presented in the publication by Gidda, S *et al.* (2003) *J. Biol. Chem.* 278, 17895-17900.

The microarray results clearly show that 12-hydroxyjasmonate induces gene expression in *A. thaliana* and that this induction is independent of the jasmonic acid induction pathway (Table 3).

To summarize, the advantages of modulating the endogenous levels of 12-hydroxyjasmonate by sulfonation over knocking down the synthesis of jasmonic acid to control flowering time are:

- The absence of negative side effects on growth.
- The absence of negative side effects on the defense response.

Hence, the Applicants submit that new claims 1 and 2 are new over the cited prior art and the Examiner is kindly requested to reconsider his rejection under U. S. C. 102(b).

In view of the above arguments and amendments, the Application is believed to be in condition for allowance.

**Table 1. Selected genes encoding enzymes involved in photosynthesis.**

<b>Accession</b>	<b>Control</b>	<b>methyl jasmonate*</b>	<b>12-hydroxyjasmonate**</b>
At1g44446	4164***	555	3810
At5g01530	42663	29136	45635
At4g27440	19087	4315	21581
At1g29910	48449	27488	53212
At1g19150	9037	6366	9204

\* Plants were treated with 50 micromolar of methyl jasmonate for a period of four hours.

\*\* Plants were treated with 50 micromolar of 12-hydroxyjasmonate for a period of four hours.

\*\*\* Numbers represent normalized gene expression values. Three independent biological replicates were used for this study.

**Table 2. Selected genes encoding proteins involved in the plant defense response**

<b>Accession</b>	<b>Control</b>	<b>methyl jasmonate</b>	<b>12-hydroxyjasmonate</b>
At5g07010	120	9744	223
At3g55970	130	6956	90
At1g63040	8	296	8
At1g72260	104	3487	64
At1g54020	71	2278	59
At5g42900	73	1905	69
At3g16330	16	363	16
At3g20810	198	3950	168
At5g37260	56	1105	50
At2g22880	8	148	9
At3g23550	247	4029	250

\* Plants were treated with 50 micromolar of methyl jasmonate for a period of four hours.

\*\* Plants were treated with 50 micromolar of 12-hydroxyjasmonate for a period of four hours.

\*\*\* Numbers represent normalized gene expression values. Three independent biological replicates were used for this study.

**Table 3. Selected genes induced by 12-hydroxyjasmonate**

<b>Accession</b>	<b>Control</b>	<b>methyl jasmonate</b>	<b>12-hydroxyjasmonate</b>
At1g30140	3	3	44
At4g04070	4	7	73
At5g21110	4	3	72

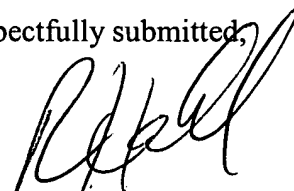
\* Plants were treated with 50 micromolar of methyl jasmonate for a period of four hours.

\*\* Plants were treated with 50 micromolar of 12-hydroxyjasmonate for a period of four hours.

\*\*\* Numbers represent normalized gene expression values. Three independent biological replicates were used for this study.

Applicants' undersigned attorney may be reached in our Washington, D.C. office by telephone at (202) 625-3500. All correspondence should continue to be directed to our address given below.

Respectfully submitted,



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Date: October 12, 2005

Attachments: Declaration

Japanese Publication No. 02-092220

Copy of USPTO date-stamped receipt

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